(12) UK Patent Application (19) GB (11) 2 327 672 (13) A

(43) Date of A Publication 03.02.1999

(21) Application No 9815170.7

(22) Date of Filing 13.07.1998

(30) Priority Data
(31) 06053536 (32) 23.07.1997 (33) US

(51) INT CL[6]
C07D 471/04 , A61K 31/47

(52) UK CL (Edition Q)
C2C CAA CKZ C1534 C1546 C213 C214 C248 C247 C25Y C250 C251 C252 C28X C282 C30Y C34Y C342 C366 C367 C575 C63X C80Y C802
C3H HA4 H302
U1S S1313 S2416

(56) Documents Cited
None

(58) Field of Search
UK CL (Edition P) C2C CKZ
INT CL[6] C07D 471/04
ONLINE:CAS-ONLINE, WPI

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(54) Abstract Title
4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-beta-alanine

(57) The compound 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine is useful for inhibiting the binding of fibrinogen to blood platelets, inhibiting the aggregation of blood platelets, inhibiting bone resorption, treating osteoporosis, inhibiting diabetic retinopathy, and inhibiting macular degeneration.

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TITLE OF THE INVENTION

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine and salts thereof

BACKGROUND OF THE INVENTION

The invention relates generally to 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine for inhibiting the binding of fibrinogen and other proteins to blood platelets, inhibiting the aggregation of blood platelets specifically to the gp IIb/IIIa fibrinogen receptor site, inhibiting bone resorption, treating and preventing osteoporosis, and inhibiting vascular restenosis, diabetic retinopathy, macular degeneration, angiogenesis, atherosclerosis, inflammation and tumor growth.

Fibrinogen is a glycoprotein present in blood plasma that participates in platelet aggregation and in fibrin formation. Platelets are cell-like anucleated fragments, found in the blood of all mammals, that also participate in blood coagulation. Interaction of fibrinogen with the IIb/IIIa receptor site is known to be essential for normal platelet function.

When a blood vessel is damaged by an injury or other causative factor, platelets adhere to the disrupted subendothethial surface. The adherent platelets subsequently release biologically active constituents and aggregate. Aggregation is initiated by the binding of agonists, such as thrombin, epinephrine, or ADP to specific platelet membrane receptors. Stimulation by agonists results in exposure of latent fibrinogen receptors on the platelet surface, and binding of fibrinogen to the glycoprotein IIb/IIIa receptor complex.

Attempts have been made to use natural products and synthetic peptides to determine the mechanism of adhesion and platelet aggregation. For example, Rouslahti and Pierschbacher in *Science, 238*, 491-497 (1987), describe adhesive proteins such as fibronectin, vitronectin, osteopontin, collagens, thrombospondin, fibrinogen, and von Willebrand factor that are present in extracellular matrices and in blood. The proteins contain the tripeptide arginine-glycine-aspartic acid

(RGD) as their glycoprotein IIb/IIIa recognition site. These arginine-glycine-aspartic acid containing tripeptides are recognized by at least one member of a family of structurally related receptors, integrins, which are heterodimeric proteins with two membrane-spanning subunits. The authors state that the conformation of the tripeptide sequence in the individual proteins may be critical to recognition specificity.

Cheresh in *Proc. Nat'l Acad. Sci. U.S.A., 84*, 6471-6475, (1987), describes an Arg-Gly-Asp directed adhesion receptor expressed by human endothethial cells that is structurally similar to the IIb/IIIa complex on platelets but is antigenically and functionally distinct. This receptor is directly involved in endothelial cell attachment to fibrinogen, von Willebrand factor, and vitronectin.

Pierschbacher and Rouslahti, in *J. of Biol. Chem., 262*, (*36*), 17294-17298 (1987) hypothesized that the Arg-Gly-Asp sequence alone would be a sufficient signal for receptor recognition and binding and that, therefore, the conformation of the tri-peptide sequence would be determinative. Various synthetic peptides were produced and the authors concluded that the stereochemical conformation of Arg-Gly-Asp as influenced by enantiomeric substitutions or additions to this sequence significantly influenced receptor-ligand interaction. The authors further showed that cyclization of a decapeptide by forming a disulfide bridge between non-terminal residues Pen and Cys, rendered the peptide much less effective at inhibiting attachment to fibronectin.

In *Proc. Nat'l Acad. Sci. U.S.A., 81*, 5985-5988 (1984), the same authors describe tetrapeptide variants of the cell recognition site of fibronectin that retain attachment-promoting activity. Peptides having a tetrapeptide recognition site are described in U.S. Pat. Nos. 4,589,881 and 4,614,517. A number of large polypeptide fragments in the cell-binding domain of fibronectin have cell-attachment activity. For example, see U.S. Pat. Nos. 4,517,686, 4,661,111 and U.S. Pat. No. 4,578,079.

Ruggeri *et al., Proc. Nat'l Acad. Sci. U.S.A., 83*, 5708-5712 (1986) explore a series of synthetic peptides designed in lengths to

16 residues, that contain RGD and a valine attached to the aspartic acid residue of RGD that inhibit fibrinogen binding to platelets. See also Koczewiak *et al., Biochem., 23,* 1767-1774 (1984); Ginsberg *et al., J. Biol. Chem., 260(7),* 3931-3936 (1985); and Haverstick *et al., Blood, 66(4),* 946-952 (1985). Other inhibitors are disclosed in Eur. Pat. App. Nos. 275,748 and 298,820.

A number of low molecular weight polypeptide factors have been isolated from snake venom. These factors apparently have high affinity for the gp IIb/IIIa complex. For example, Huang *et al., J. Biol Chem., 262,* 16157-16163 (1987); Huang *et al., Biochemistry, 28,* 661-666 (1989) describe the primary structure of the venom trigramin which is a 72 amino acid polypeptide that contains the RGD subunit. Echistatin is another compound which has high affinity for the gp IIb/IIIa complex. This polypeptide contains 49 amino acids and has the RGD subunit and various disulfide bridges. Gan *et al., J. Biol. Chem., 263,* 19827-19832 (1988). See also, Dennis *et al., Proc. Nat'l Acad. Sci. USA, 87,* 2471-2475 (1989). However, these snake venom factors also have high affinity for other members of the adhesive protein receptor family including the vitronectin and fibronectin receptors so are not selective for the gp IIb/IIIa complex.

While it is known that the tripeptide sequence Arg-Gly-Asp is present in certain polypeptides that can duplicate or inhibit the cell attachment-promoting effects of fibronectin and vitronectin, the tripeptide Arg-Gly-Asp has low activity. At present, there is little understanding of how other amino acids coupled to this sequence influence binding specificity. U.S. Pat. No 5,023,233, assigned to Merck & Co., Inc., discloses small cyclic hexapeptides which contain the sequence Arg-Gly-Asp and are useful platelet aggregation inhibitors. U.S. Pat. No. 5,037,808 discloses the use of indolyl platelet-aggregation inhibitors which are believed to act by antagonizing interactions between fibrinogen and/or extracellular matrix proteins and the platelet gp IIb/IIIa receptor. U.S. Pat. No. 5,037,808 discloses guanidino peptide mimetic compounds that retain an Asp residue which inhibit platelet aggregation. The application PCT/US90/02746 describes the use of

antibody-poly-peptide conjugates wherein said polypeptides contain the Arg-Gly-Asp (RGD) sequence.

The application PCT/US91/00564 discloses the use of large cyclic peptides containing RGD flanked by proline residues which are platelet aggregation inhibitors. The application PCT/US90/03788 discloses small cyclic platelet aggregation inhibitors which are synthetic cyclic pentapeptides containing the tripeptide sequence Arg-Gly-Asp and a thioether linkage in the cycle. The application PCT/US90/05367 published May 2, 1991 also discloses the use of peptides and pseudopeptides such as N-amidino-piperidine-3-carboxylglycyl-L-aspartyl-L-valine that inhibit platelet aggregation and thrombus formation in mammalian blood. The application Eur. Pat. App. No. 91103462.7 discloses linear compounds which can include internal piperazinyl or piperidinyl derivatives. Eur. Pat. App. No. 91300179.8, assigned to Merck & Co., Inc., and published on July 17, 1991 discloses linear polypeptide fibrinogen receptor antagonists. Eur. Pat. App. No. 90101404.3 discloses compounds of the R^1-A-$(W)_a$-X-$(CH_2)_b$-$(Y)_c$-B-Z-COOR wherein R^1 is a guandidino or amidino moiety and A and B are chosen from specific monosubstituted aryl or heterocyclic moieties.

Bone resorption is mediated by the action of a class of cells known as osteoclasts. Osteoclasts are multinucleated cells of up to 400 μm in diameter that resorb mineralized tissue, chiefly calcium carbonate and calcium phosphate, in vertebrates. They are actively motile cells that migrate along the surface of bone. They can bind to bone, secrete necessary acids and proteases and thereby cause the actual resorption of mineralized tissue from the bone.

More specifically, osteoclasts are believed to exist in at least two physiological states. In the secretory state, osteoclasts are flat, attach to the bone matrix via a tight attachment zone (sealing zone), become highly polarized, form a ruffled border, and secrete lysosomal enzymes and protons to resorb bone. The adhesion of osteoclasts to bone surfaces is an important initial step in bone resorption. In the migratory or motile state, the osteoclasts migrate across bone matrix and do not take part in resorption until they attach again to bone.

Integrins are transmembrane, heterodimeric, glycoproteins which interact with extracellular matrix and are involved in osteoclast attachment, activation and migration. The most abundant integrin in osteoclasts (rat, chicken, mouse and human) is the vitronectin receptor, or $\alpha v\beta 3$, thought to interact in bone with matrix proteins that contain the RGD sequence. Antibodies to $\alpha v\beta 3$ block bone resorption in vitro indicating that this integrin plays a key role in the resorptive process. There is increasing evidence to suggest that $\alpha v\beta 3$ ligands can be used effectively to inhibit osteoclast mediated bone resorption in vivo in mammals.

The current major bone diseases of public concern are osteoporosis, hypercalcemia of malignancy, osteopenia due to bone metastases, periodontal disease, hyperparathyroidism, periarticular erosions in rheumatoid arthritis, Paget's disease, immobilization-induced osteopenia, and glucocorticoid treatment.

All these conditions are characterized by bone loss, resulting from an imbalance between bone resorption (breakdown) and bone formation, which continues throughout life at the rate of about 14% per year on the average. However, the rate of bone turnover differs from site to site, for example, it is higher in the trabecular bone of the vertebrae and the alveolar bone in the jaws than in the cortices of the long bones. The potential for bone loss is directly related to turnover and can amount to over 5% per year in vertebrae immediately following menopause, a condition which leads to increased fracture risk.

There are currently 20 million people with detectable fractures of the vertebrae due to osteoporosis in the United States. In addition, there are 250,000 hip fractures per year attributed to osteoporosis. This clinical situation is associated with a 12% mortality rate within the first two years, while 30% of the patients require nursing home care after the fracture.

Individuals suffering from all the conditions listed above would benefit from treatment with agents which inhibit bone resorption.

Additionally, $\alpha v\beta 3$ ligands have been found to be useful in treating and/or inhibiting restenosis (recurrence of stenosis after

corrective surgery on the heart valve), atherosclerosis, diabetic retinopathy, macular degeneration and angiogenesis (formation of new blood vessels). Moreover, it has been postulated that the growth of tumors depends on an adequate blood supply, which in turn is dependent on the growth of new vessels into the tumor; thus, inhibition of angiogenesis can cause tumor regression in animal models. (*See*, Harrison's Principles of Internal Medicine, 12th ed., 1991). $\alpha v\beta 3$ antagonists, which inhibit angiogenesis, are therefore useful in the treatment of cancer for inhibiting tumor growth. (*See e.g.*, Brooks et al., *Cell*, 79:1157-1164 (1994)).

Moreover, the compound of this invention can also inhibit neovascularization by acting as antagonists of the integrin receptor $\alpha v\beta 5$. A monoclonal antibody for $\alpha v\beta 5$ has been shown to inhibit VEGF-induced angiogenesis in rabbit cornea and the chick chorioallantoic membrane model; M.C. Friedlander, et al., *Science* 270, 1500-1502, 1995. Thus, compounds that antagonize $\alpha v\beta 5$ are useful for treating and preventing macular degeneration, diabetic retinopathy, and tumor growth.

In addition, the compound of this invention antagonizes both the $\alpha v\beta 3$ and $\alpha v\beta 5$ receptors. The compound, referred to as a "dual $\alpha v\beta 3/\alpha v\beta 5$ antagonists," is useful for inhibiting bone resorption, treating and preventing osteoporosis, and inhibiting vascular restenosis, diabetic retinopathy, macular degeneration, angiogenesis, atherosclerosis, inflammation and tumor growth.

While a multitude of compounds or peptide analogs believed to inhibit platelet aggregation by inhibiting binding to a blood platelet by fibrinogen are known, the present invention provides a compound that has significant binding activity and is, therefore, useful for the reasons stated herein. A number of very serious diseases and disorders involve hyperthrombotic complications which lead to intravascular thrombi and emboli. Myocardial infarction, stroke, phlebitis and a number of other serious conditions create the need for novel and effective fibrinogen receptor antagonists.

The compound of the present invention is also useful for preventing or reducing the incidence of osteoporosis. Additionally, it has been found that the compound is also useful for treating and/or inhibiting restenosis, diabetic retinopathy, macular degeneration, atherosclerosis and/or angiogenesis in mammals.

SUMMARY OF THE INVENTION

The invention is 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine and pharmaceutically acceptable salts thereof.

The compound of the invention is useful in a method for eliciting an $\alpha_v\beta_3$, $\alpha_v\beta_5$ or dual $\alpha_v\beta_3/\alpha_v\beta_5$ antagonizing effect in a mammal in need thereof, which involves administering to the mammal a therapeutically effective amount of the compound.

The compound of the invention is also useful for inhibiting the bone resorption activity of mammalian osteoclast cells by administering a pharmacologically effective amount of the compound to a patient in need of such activity to inhibit the activity of mammalian osteoclasts.

The compound of the invention is also useful for inhibiting tumor growth in mammals. Pharmacologically effective amounts of the compound, including pharmaceutically acceptable salts thereof, are administered to the mammal, to inhibit tumor growth. The growth of tumors depends on an adequate blood supply, which in turn depends on growth of new vessels into the tumor. New vessels are stimulated by factors secreted by the tumor. Inhibition of angiogenesis can cause tumor regression in animals.

The compound of the invention is also useful for treating and preventing diabetic retinopathy in mammals. Pharmacologically effective amounts of the compound, including pharamaceutically acceptable salts thereof, are administered to the mammal, to inhibit diabetic retinopathy.

The compound of the invention is also useful in the prevention of restenosis of vessels.

Exemplifying the invention is the method wherein the antagonizing effect is an αvβ3 antagonizing effect illustrated by the effects of inhibiting bone resorption, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth.

Another example of the invention is the method wherein the antagonizing effect is an αvβ5 antagonizing effect illustrated by the effects of inhibiting restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth.

Another example of the invention is the method wherein the antagonizing effect is a dual αvβ3/αvβ5 antagonizing effect illustrated by the effect of inhibiting bone resorption, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth.

More particularly illustrating the invention is a pharmaceutical composition comprising 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine and a pharmaceutically acceptable carrier. Another example of the invention is a pharmaceutical composition made by combining 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine and a pharmaceutically acceptable carrier. Another illustration of the invention is a process for making a pharmaceutical composition comprising combining 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine and a pharmaceutically acceptable carrier.

Further illustrating the invention is a method of treating and/or preventing a condition mediated by antagonism of a vitronectin receptor in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine. Preferably, the condition is selected from bone resorption, osteoporosis, restenosis, diabetic retinopathy, macular degeneration, angiogenesis, atherosclerosis, inflammation, cancer and tumor growth. More preferably, the condition is selected from osteoporosis and cancer. Most preferably, the condition is osteoporosis.

More specifically exemplifying the invention is a method of eliciting an $\alpha_v\beta_3$, $\alpha_v\beta_5$ or dual $\alpha_v\beta_3/\alpha_v\beta_5$ antagonizing effect in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine or any of the pharmaceutical compositions described above. Preferably, the antagonizing effect is an $\alpha v\beta 3$ antagonizing effect; more specifically the $\alpha v\beta 3$ antagonizing effect is selected from inhibition of bone resorption, inhibition of restenosis, inhibition of atherosclerosis, inhibition of aniogenesis, inhibition of diabetic retinopathy, inhibition of macular degeneration, inhibition of inflammation or inhibition of tumor growth. Alternatively, the antagonizing effect is an $\alpha v\beta 5$ antagonizing effect (e.g. inhibition of restenosis, atherosclerosis, aniogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth) or a dual $\alpha v\beta 3/\alpha v\beta 5$ antagonizing effect (e.g. inhibition of bone resorption, restenosis, atherosclerosis, aniogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth).

Additional examples of the invention are methods of treating and/or preventing osteoporosis in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine or any of the pharmaceutical compositions described above.

Further exemplifying the invention is any of the compositions described above, further comprising a therapeutically effective amount of a second bone resorption inhibitor; preferably, the second bone resorption inhibitor is alendronate.

More particularly illustrating the invention is any of the methods of treating and/or preventing osteoporosis and/or of inhibiting bone resorption described above, wherein 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine is administered in combination with a second bone resorption inhibitor; preferably, the second bone resorption inhibitor is alendronate.

Additional illustrations of the invention are methods of treating hypercalcemia of malignancy, osteopenia due to bone metastases, periodontal disease, hyperparathyroidism, periarticular erosions in rheumatoid arthritis, Paget's disease, immobilization-induced osteopenia, and glucocorticoid treatment in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine or any of the pharmaceutical compositions described above.

More particularly exemplifying the invention is the use of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine in the preparation of a medicament for the treatment and/or prevention of osteoporosis in a mammal in need thereof. Still further exemplifying the invention is the use of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine in the preparation of a medicament for the treatment and/or prevention of: bone resorption, tumor growth, cancer, restenosis, atherosclerosis, diabetic retinopathy, macular degeneration, inflammation and/or angiogenesis.

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine is useful for inhibiting the binding of fibrinogen to blood platelets and for inhibiting the aggregation of blood platelets. 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine can be used in a method of acting upon a fibrinogen receptor which comprises administering a therapeutically effective but non-toxic amount of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine to a mammal, preferably a human. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, dispersed therein, an effective but non-toxic amount of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine is another feature of this invention.

The invention also includes the use of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-

alanine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inhibiting the aggregation of blood platelets, preventing platelet thrombosis, preventing thromboembolism or preventing reocclusion, in a mammal.

DETAILED DESCRIPTION OF THE INVENTION

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine is useful in a method of inhibiting the binding of fibrinogen to blood platelets and for inhibiting the aggregation of blood platelets.

One test which is used to evaluate fibrinogen receptor antagonist activity is based on evaluation of inhibition of ADP-stimulated platelets. Aggregation requires that fibrinogen bind to and occupy the platelet fibrinogen receptor site. Inhibitors of fibrinogen binding inhibit aggregation. In the ADP-stimulated platelet aggregation assay used to determine inhibition associated with 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine, human platelets are isolated from fresh blood, collected into acid citrate/dextrose by differential centrifugation followed by gel filtration on Sepharose 2B in divalent ion-free Tyrode's buffer (pH 7.4) containing 2% bovine serum albumin.

Platelet aggregation is measured at 37°C in a Chronolog aggregometer. The reaction mixture contains gel-filtered human platelets (2 x 10^8 per ml), fibrinogen (100 micrograms per ml (ug/ml)), Ca^{2+} (1 mM), and the compound to be tested. The aggregation is initiated by adding 10 mM ADP 1 minute after the other components are added. The reaction is then allowed to proceed for at least 2 minutes. The extent of inhibition of aggregation is expressed as the percentage of the rate of aggregation observed in the absence of inhibitor. The IC_{50} is the dose of a particular compound inhibiting aggregation by 50% relative to a control lacking the compound.

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine displays submicromolar affinity for the human αvβ3 receptor. 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-

yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine is therefore useful for treating mammals suffering from a bone condition caused or mediated by increased bone resorption, who are in need of such therapy. Pharmacologically effective amounts of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine, including pharamaceutically acceptable salts thereof, are administered to the mammal, to inhibit the activity of mammalian osteoclasts.

The term "pharmaceutically acceptable salts" shall mean non-toxic salts of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts include the following salts: acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, trifluoroacetate, and valerate.

The compound of the present invention is chiral; included within the scope of the present invention are racemic mixtures and separated enantiomers of the compound. Furthermore, all diastereomers, including E, Z isomers, of the compound are included in the present scope. Furthermore, hydrates as well as anhydrous compositions and polymorphs of the compound are within the present invention.

Prodrugs, such as ester derivatives of described compound, are compound derivatives which, when absorbed into the bloodstream of a warm-blooded animal, cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy. The

present invention includes within its scope prodrugs of the compound of this invention. In general, such prodrugs will be functional derivatives of the compound of this invention which are readily convertible in vivo into the required compound. Thus, in the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various conditions described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound in vivo after administration to the patient. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs," ed. H. Bundgaard, Elsevier, 1985. Metabolites of the compound include active species produced upon introduction of compound of this invention into the biological milieu.

The term "pharmaceutically effective amount" shall mean that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system or animal that is being sought by a researcher or clinician.

The term "anti-coagulant" shall include, but not be limited to, heparin, and warfarin.

The term "thrombolytic agent" shall include, but not be limited to, agents such as streptokinase and tissue plasminogen activator.

The term "platelet anti-aggregation agent" shall include, but not be limited to, agents such as aspirin and dipyridamole.

The terms "$\alpha_v\beta_3$ receptor antagonist", "$\alpha_v\beta_5$ receptor antagonist" or "dual $\alpha_v\beta_3/\alpha_v\beta_5$ receptor antagonist," as used herein, refers to a compound which binds to and antagonizes either the $\alpha_v\beta_3$ receptor or the $\alpha_v\beta_5$ receptor, or a compound which binds to and antagonizes both the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors (i.e., a dual $\alpha_v\beta_3/\alpha_v\beta_5$ receptor antagonist).

The term "bone resorption" means the process by which osteoclasts solubilize bone minerals and increase the activity of enzymes that degrade bone matrix.

In the schemes and examples below, various reagent symbols have the following meanings:

BOC (or Boc):	t-butyloxycarbonyl
Pd/C:	Palladium on activated carbon catalyst
DMF:	Dimethylformamide
EtOH:	ethanol
EtOAc:	ethyl acetate
EDC:	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
NH_4Ac:	ammonium acetate
HOBT:	1-hydroxybenzotriazole hydrate
NMM:	N-methyl morpholine
$NaHCO_3$:	sodium hydrogen carbonate
$MgSO_4$:	magnesium sulfate
NaOH:	sodium hydroxide
NH_4OH:	ammonium hydroxide
$((CH_3)_3Sn)_2$:	hexamethylditin
$Pd(PPh_3)_4$:	tetrakis(triphenylphosphine)palladium

The compound of the present invention can be administered in such oral forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. Likewise, it may be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, ocular or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as an anti-aggregation agent.

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine may be administered to patients where prevention of thrombosis by inhibiting binding of fibrinogen to the platelet membrane glycoprotein complex IIb/IIIa receptor is desired. It is useful in surgery on peripheral arteries (arterial grafts, carotid endarterectomy) and in cardiovascular surgery where manipulation of

arteries and organs, and/or the interaction of platelets with artificial surfaces, leads to platelet aggregation and consumption. The aggregated platelets may form thrombi and thromboemboli. 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine may be administered to these surgical patients to prevent the formation of thrombi and thromboemboli.

Extracorporeal circulation is routinely used for cardiovascular surgery in order to oxygenate blood. Platelets adhere to surfaces of the extracorporeal circuit. Adhesion is dependent on the interaction between gp IIb/IIIa on the platelet membranes and fibrinogen adsorbed to the surface of the circuit. (Gluszko *et al.*, *Amer. J. Physiol.*, *252*(H), 615-621 (1987)). Platelets released from artificial surfaces show impaired hemostatic function. 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine may be administered to prevent adhesion.

Other applications of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine include prevention of platelet thrombosis, thromboembolism and reocclusion during and after thrombolytic therapy and prevention of platelet thrombosis, thromboembolism and reocclusion after angioplasty or coronary artery bypass procedures. It may also be used to prevent myocardial infarction.

The dosage regimen utilizing the compound of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and whether the compound or a particular salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

Oral dosages of the present invention, when used for the indicated effects, will range between about 0.01 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day and preferably

0.01-50 mg/kg/day and more preferably 0.01-20 mg/kg/day, e.g. 0.1 mg/kg/day, 1.0 mg/kg/day, 5.0 mg/kg/day, or 10 mg/kg/day. A once-a-day oral dosage is, for example, 10 mg, 100 mg, or 500 mg.

Intravenously, the most preferred doses will range from about 1 to about 10 mg/kg/minute during a constant rate infusion. Advantageously, 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine may be administered in divided doses of two, three, or four times daily. Furthermore, the compound can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, or course, be continuous rather that intermittent throughout the dosage regime.

In ocular formulations such as eyedrops (e.g. aqueous solutions), from about 0.01-5.0% (w/v) of active ingredient can be employed, e.g., from about 0.01-2.0% (w/v) of active ingredient. Suitable eyedrop volume is, for example, 20, 30, 35, 50 or 100 µl. The objective is to administer a dose of between about 0.005-0.5 mg/kg per day to each eye, for a total dosage of between about 0.01-1.0 mg/kg/day, e.g. a dose of about 0.05 mg/kg per day to each eye, for a total dosage of about 0.1 mg/kg/day. For example, the eyedrops can be used to provide doses of 1 mg, 10 mg, or 50 mg. These dosage values are based on known and presently understood pharmacology of compound of the invention. Dosage requirements are variable and must be individualized on the basis of the disease and the response of the patient.

Suitable eyedrop formulations are those which are isotonic and maintain sufficient contact with the eye surface to systemically deliver the active agent to the patient. Such formulations advantageously have a pH approximating neutrality and are non-irritating to the eye, e.g. they do not induce tearing and consequential flow of active agent out of the eye. Pharmaceutically acceptable carriers are, for example, water, mixtures of water and water-miscible solvents such as lower alkanols or arylalkanols, vegetable oils,

polyalkylene glycols, petroleum based jelly, ethyl cellulose, hydroxy ethyl cellulose, ethyl oleate, carboxymethylcellulose, polyvinylpyrrolidone, isopropyl myristate and other conventionally-employed non-toxic, pharmaceutically acceptable organic and inorganic carriers. The pharmaceutical preparation may also contain non-toxic auxiliary substances such as emulsifying, preserving, wetting agents, bodying agents and the like, as for example, polyethylene glycols 200, 300, 400 and 600, carbowaxes 1000, 1500, 4000, 6000 and 10000, antibacterial compounds, phenylmercuric salts known to have cold sterilizing properties and which are non-injurious in use, thimerosal, methyl and propyl paraben, benzyl alcohol, phenyl ethanol, buffering ingredients such as sodium chloride, sodium borate, sodium acetates, gluconate buffers, and other conventional ingredients such as sorbitan monolaurate, triethanolamine, oleate, polyoxyethylene sorbitan monopalmitylate, dioctyl sodium sulfosuccinate, monothioglycerol, thiosorbitol, ethylenediamine tetraacetic acid, and the like. Additionally, suitable ophthalmic vehicles can be used as carrier media for the present purpose including conventional phosphate buffer vehicle systems, isotonic boric acid vehicles, isotonic sodium chloride vehicles, isotonic sodium borate vehicles and the like.

In the procedure for making eyedrops, formulations are rendered sterile by appropriate means, such as starting the preparation procedure with sterile components and proceeding under sterile conditions, irradiating or autoclaving the finished formulation, and the like. Suitable anti microbial agents are also useful for maintaining sterility of the eyedrop.

The ocular preparation may also be a solid insert such as one which, after dispensing the compound, remains essentially intact, or a bioerodible insert that is soluble in lacrimal fluids, or otherwise disintegrates. For example, one may use a solid water soluble polymer as the carrier for the compound. The polymer used to form the insert may be any water soluble non-toxic polymer, for example, cellulose derivatives such as methylcellulose, sodium carboxymethyl cellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethyl

cellulose, acrylates such as polyacrylic acid salts, ethylacrylates, polyacrylamides, natural products such as gelatin, alginates, pectins, tragacanth, karaya, chondrus, agar, acacia, starch derivatives such as starch acetate, hydroxyethyl starch ethers, hydroxypropyl starch, as well as other synthetic derivatives such as polyvinyl alcohol, polyvinyl pyrrolidone, polyvinyl methyl ether, polyethylene oxide, neutralized carbopol, gellan gum and xanthan gum, and mixtures of said polymers.

The ocular preparation may also be an ointment which is compounded, for example, by mixing finely milled powdered ingredients with a small amount of white petrolatum and levigating or otherwise mixing until a uniform distribution is achieved. The balance of white petrolatum is added by geometric addition until the desired dosage form is made.

In the methods of the present invention, the compound can form the active ingredient, and is typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with convention pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the compound can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn-sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms

include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch methyl cellulose, agar, bentonite, xanthan gum and the like.

The compound of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

The compound of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound is coupled. The compound of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxy-ethyl-aspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the compound of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels.

The compound of the present invention can also be co-administered with suitable anticoagulation agents or thrombolytic agents such as plasminogen activators or streptokinase to achieve synergistic effects in the treatment of various vascular pathologies. It may also be combined with heparin, aspirin, or warfarin.

The present invention is also directed to combinations of the compound of the present invention with one or more agents useful in the prevention or treatment of osteoporosis. For example, the compound of the instant invention may be effectively administered in combination with effective amounts of other agents used in the treatment of osteoporosis such as bisphosphonate bone resorption inhibitors;

preferably, the bone resorption inhibitor is the bisphosphonate alendronate, now sold as FOSAMAX®. Preferred combinations are simultaneous or alternating treatments of an αvβ3 receptor antagonist of the present invention and FOSAMAX®.

In addition, the compound of the present invention may be effectively administered in combination with a growth hormone secretagogue in the therapeutic or prophylactic treatment of disorders in calcium or phosphate metabolism and associated diseases. These diseases include conditions which can benefit from a reduction in bone resorption. A reduction in bone resorption should improve the balance between resorption and formation, reduce bone loss or result in bone augmentation. A reduction in bone resorption can alleviate the pain associated with osteolytic lesions and reduce the incidence and/or growth of those lesions. These diseases include: osteoporosis (including estrogen deficiency, immobilization, glucocorticoid induced and senile), osteodystrophy, Paget's disease, myositis ossificans, Bechterew's disease, malignant hypercalcemia, metastatic bone disease, periodontal disease, cholelithiasis, nephrolithiasis, urolithiasis, urinary calculus, hardening of the arteries (sclerosis), arthritis, bursitis, neuritis and tetany. Increased bone resorption can be accompanied by pathologically high calcium and phosphate concentrations in the plasma, which would be alleviated by this treatment. Similarly, the present invention would be useful in increasing bone mass in patients with growth hormone deficiency. Thus, preferred combinations are simultaneous or alternating treatments of an αvβ3 receptor antagonist of the present invention and a growth hormone secretagogue, optionally including a third component comprising FOSAMAX®.

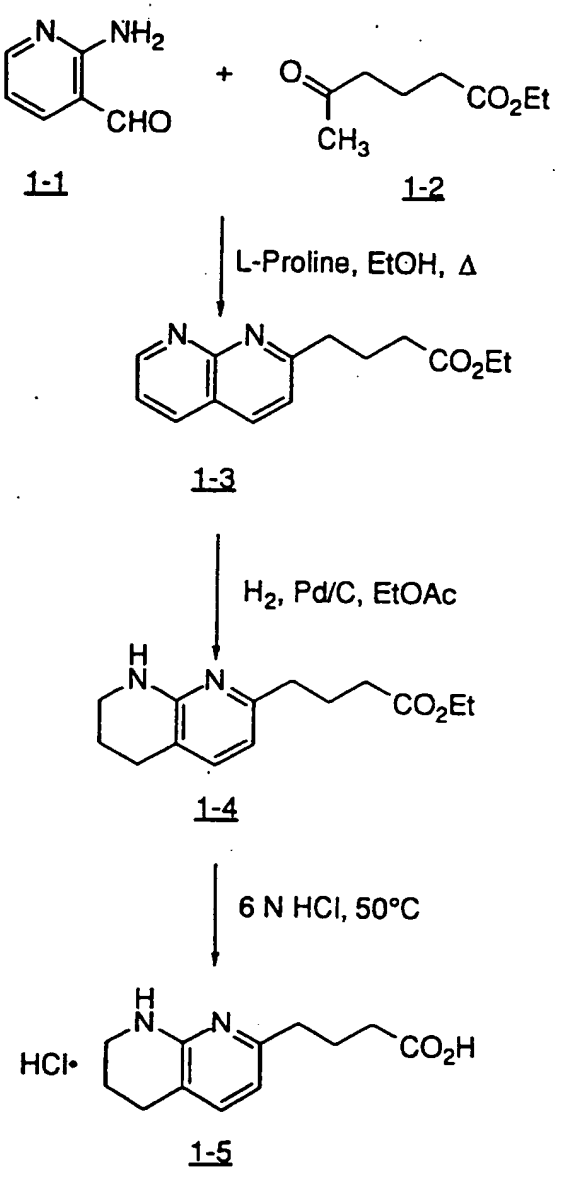
It will be understood that the scope of combinations of the compound of this invention with other agents useful for treating αvβ3 related conditions includes in principle any combination with any pharmaceutical composition useful for treating osteoporosis.

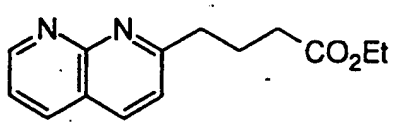
In accordance with the method of the present invention, the individual components of the combination can be administered separately at different times during the course of therapy or

concurrently in divided or single combination forms. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine was prepared according to the procedure of the following example. The example further illustrates details for the preparation of the compound of the present invention. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare this compound. All temperatures are degrees Celsius unless otherwise noted.

SCHEME 1


NH2
CHO
+
O
CO2Et
CH3
1-1
1-2
L-Proline, EtOH, Δ
N
N
CO2Et
1-3
H2, Pd/C, EtOAc
H
N
N
CO2Et
1-4
6 N HCl, 50°C
H
N
N
CO2H
HCl•
1-5



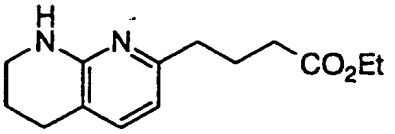
1-3

Ethyl 4-(1,8-naphthyridin-2-yl)butanoate (1-3)

Aminoaldehyde 1-1 (2.02 g, 16.6 mmol, prepared according to *Het.* 1993, *36*, 2513), ketone 1-2 (5.3 mL, 33.1 mmol) and L-proline (0.48 g, 4.17 mmol) were combined in 75 mL EtOH. After heating at reflux overnight the reaction was concentrated. Flash chromatography (silica, EtOAc) provided 1-3 as an off-white crystalline solid.

TLC R_f 0.23 (silica, EtOAc)

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.09 (dd, J=4, 2Hz, 1H), 8.17 (dd, J=8, 2Hz, 1H), 8.12 (d, J=8Hz, 1H), 7.46 (dd, J=8, 4Hz, 1H), 7.42 (d, J=8Hz, 1H), 4.12 (q, J=7Hz, 2H), 3.11 (t, J=8Hz, 2H), 2.44 (t, J=7Hz, 1H), 2.26 (qn, J=8Hz, 2H), 1.25 (t, J=7Hz, 3H).



1-4

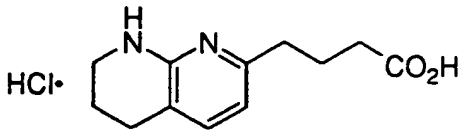
Ethyl 4-(1,2,3,4-tetrahydro-1,8-naphthyridin-7-yl)butanoate (1-4)

A solution of 1-3 (2.3 g, 9.4 mmol) in 50 mL EtOAc was treated with 10% Pd/C (230 mg) and a hydrogen balloon. After 4 d the reaction filtered through celite, concentrated, and purified by flash chromatography (silica, 70% EtOAc/hexane), providing 1-4 as a yellow oil.

TLC R_f 0.40 (silica, EtOAc)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05 (d, J=7Hz, 1H), 6.35 (d, J=7Hz, 1H), 4.73 (br s, 1H), 4.12 (q, J=7Hz, 2H), 2.69 (t, J=6Hz, 2H), 2.57 (t,

J=8Hz, 2H), 2.33 (t, J=7Hz, 2H), 1.98 (m, 2H), 1.90 (m, 2H), 1.25 (t, J=7Hz, 3H).

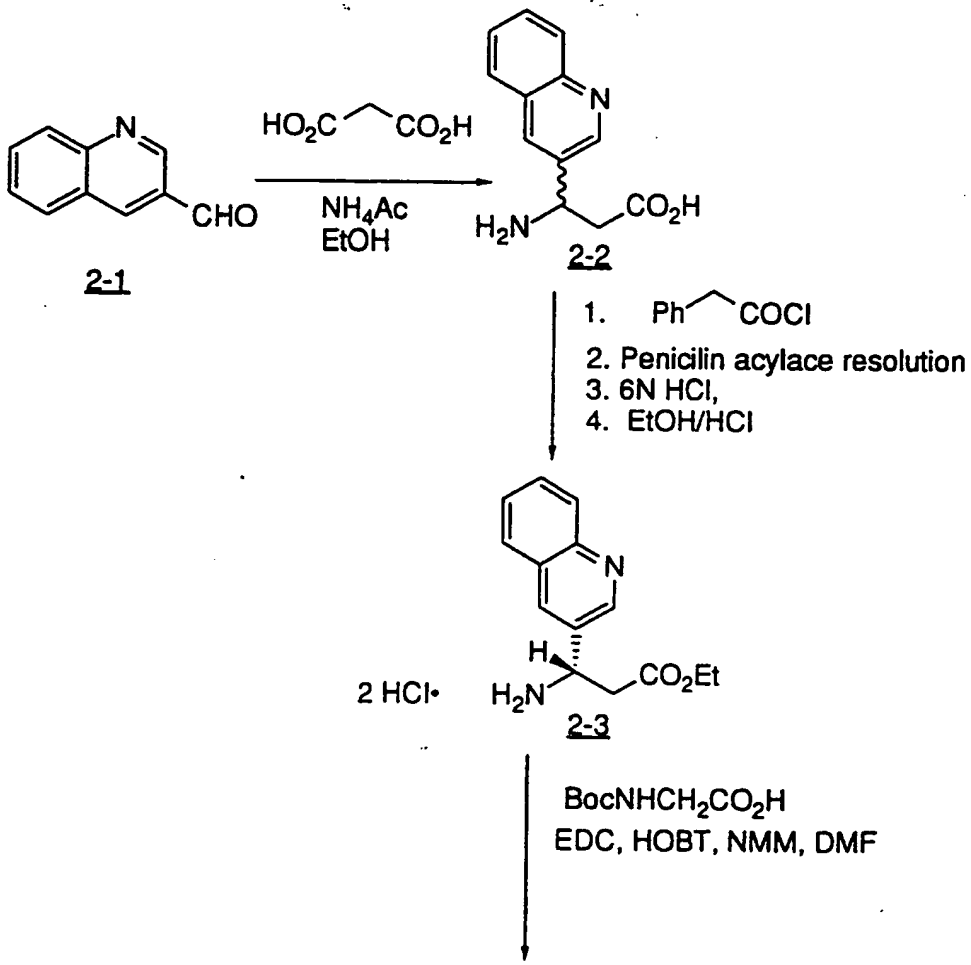


1-5

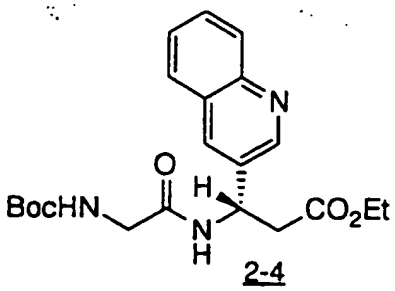
4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoic acid hydrochloride (1-5)

Ester 1-4 (1.8 g, 7.25 mmol) in 36 mL 6 N HCl was heated at 50°C for 4 h, then concentrated, providing 1-5 as a yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.59 (d, J=7Hz, 1H), 6.63 (d, J=7Hz, 1H), 3.50 (t, J=5Hz, 2H), 2.82 (t, J=6Hz, 2H), 2.74 (t, J=8Hz, 2H), 2.38 (t, J=7Hz, 2H), 2.02-1.90 (m, 4H).

SCHEME 2

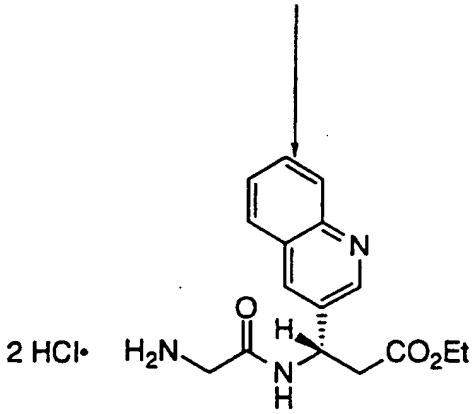

N
CHO
2-1
HO2C
CO2H
NH4Ac
EtOH
N
H2N
CO2H
2-2
1. Ph COCl
2. Penicilin acylace resolution
3. 6N HCl,
4. EtOH/HCl
N
H
2 HCl•
H2N
CO2Et
2-3
BocNHCH2CO2H
EDC, HOBT, NMM, DMF

SCHEME 2 (CONT'D)



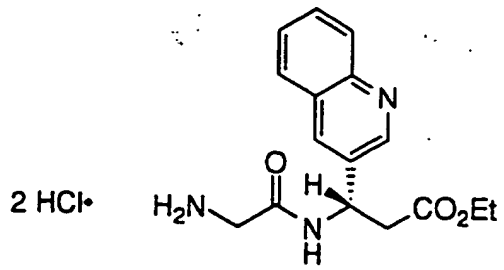
2-4

HCl, EtOH

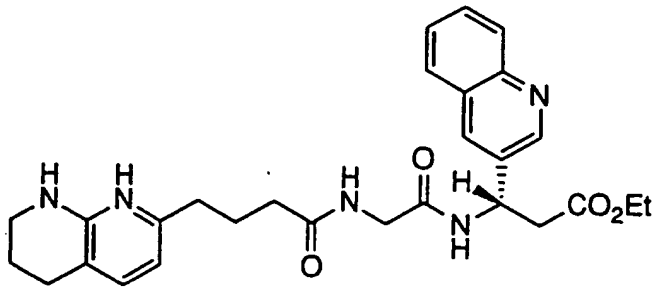


2-5

SCHEME 2 (CONT'D)

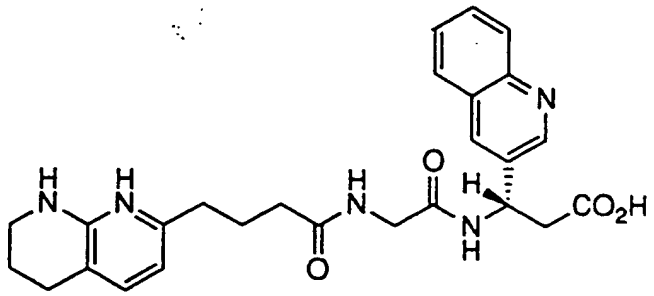


2-5



2-6

SCHEME 2 (CONT'D)



2-7

3(S)-Quinolin-3-yl-β-alanine ethyl ester hydrochloride (2-3)

A solution containing 2-1 3-carboxaldehyde (5 g, 31.8 mmol), malonic acid (3.6 g, 35.0 mmol), and ammonium acetate (5.0 g, 63.6 mmol) in anhydrous ethanol (125 mL) was heated at reflux for 12 h. After cooling to room temperature, the resulting white solid was collected by filtration and washed with cold ethanol (50 mL) and then dried under vacuum to provide 2-2 as a white solid.

^{1}H NMR (300 MHz, D_2O): δ 8.91 (d, J = 2 Hz 1H), 8.21 (d, J = 2Hz, 1H), 8.12 (d, J = 8Hz, 1H), 7.84 (d, J = 7 Hz, 1H), 7.72 (t, J= 7Hz, 1H), 7.54 (t, J = 7 Hz, 1,H), 4.72 (M, 1H), 2.73 (M, 2H). The (S)-enantiomer of 25-2 was prepared using the enzymatic resolution described by Soloshonok et al. (Tetrahedron: Asymmetry, 6, 1601, *1995*). The resolved material was converted to 25-3 by refluxing in ethanolic HCl.

^{1}H NMR (300 MHz, CD_3OD): δ 9.25 (d, J = 2Hz 1H), 8.31 (d, J = 2Hz, 1H), 8.15 (d, J = 8Hz, 1H), 7.84 (d, J =7 Hz, 1H), 7.72 (t, J = 7Hz, 1H), 7.54 (t, J = 7 Hz, 1,H), 4.72 (M, 1H), 4.15 (q, J = 6 Hz, 2H), 2.73 (M, 2H) 1.18 (t, J= 6 Hz, 3H).

N-Boc-Glycyl-3(S)-quinolin-3-yl-β-alanine ethyl ester (2-4)

N-Boc-Glycine (60.7 mg, 0.35 mmol), amine 2-3 (0.10 mg, 3.15 mmol), NMM (0.13 mL, 1.26 mmol) and EDC (78.5 mg, 0.41 mmol) were combined in 3 mL DMF. After stirring overnight the mixture was concentrated, diluted with EtOAc, washed with water, sat.

$NaHCO_3$, water, and brine, dried ($MgSO_4$), filtered and concentrated. Flash chromatography (silica, EtOAc) provided 2-4 as a colorless oil. TLC R_f 0.45 (silica, EtOAc).

^1H NMR (300 MHz, $CDCl_3$): δ 8.88 (d, J = 2Hz 1H), 8.09 (d, J = 2Hz, 1H), 8.05 (d, J = 8Hz, 1H), 7.79 (d, J =6 Hz, 1H), 7.72 (t, J = 6Hz, 1H), 7.68 (br d, 1H), 7.54 (t, J = 7 Hz, 1,H), 5.65 (M, 1H), 5.18 (br, t, 1H), 4.15 (q, J = 6 Hz, 2H), 3.00 (M, 2H), 1.21 (s, 9H), 1.08 (t, J= 7 Hz, 3H).

Glycyl-3(S)-quinolin-3-yl-β-alanine dihydrochloride (2-5)

The ester 2-4 (92 mg, 0.23 mmol) was dissolved in HCl saturated ethanol and stirred at room temperature for 3.5 h then concentrated at reduced pressure to afford 2-5 as a white solid.

^1H NMR (300 MHz, CD_3OD): δ 9.38 (s, 1H), 9.15 (s, 1H), 8.45 (d, J = 6Hz, 1H), 8.22 (d, J = 6 Hz, 1H), 8.17 (t, J = 6Hz, 1H), 8.00 (t, J = 7 Hz, 1,H), 5.65 (M, 1H), 5.18 (br, t, 1H), 4.15 (q, J = 6 Hz, 2H), 3.00 (M, 2H), 1.21 (s, 9H), 1.08 (t, J= 7 Hz, 3H).

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine ethyl ester (2-6)

A mixture of 1-5 (57 mg, 0.22 mmol), 2-5 (84 mg, 0.22 mmol), NMM (123 mL, 1.12 mmol) and, HOBT (39 mg, 0.29 mmol) and EDC (55 mg, 0.29 mmol) in 2 mL DMF was stirred overnight. After diluting with EtOAc the mixture was washed with sat. $NaHCO_3$, water, and brine, dried ($MgSO_4$), filtered and concentrated, and chromatographed on silica (30% MeOH/EtOAc) providing 2-6.

^1H NMR (300 MHz, $CDCl_3$): δ 9.91(s, 1H), 8.56 (br t, 1H), 8.18 (br d, 1H), 8.16 (s, 1H), 8.05 (d, J = 6 Hz, 1H), 7.82 (d, J= 6 Hz, 1H), 7.73 (t, J = 6 Hz, 1H), 7.54 (t, J = 6 Hz, 1H), 7.08 (d, J = 7Hz, 1H), 6.33 (d, J = 7Hz, 1H), 5.71 (m, 1H), 5.69 (br s, 1H), 4.15 (d, J = 7Hz, 2H), 4.05, (t, J = 7 Hz, 2H), 3.53 (q, J=6Hz, 2H), 3.43 (m, 2H), 3.00 (m, 2H), 2.69 (t, J = 6Hz, 2H), 2.60 (t, J = 7Hz, 2H), 2.46 (t, J = 6Hz, 2H), 2.25 (t, J = 7Hz, 2H), 2.05-1.90 (m, 4H), 1.08 (t, J = 7 Hz, 3H).

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine (2-7)

Ester 2-6 was dissolved in 5 mL 6N HCl and warmed to 50°C for 1.5 h then concentrated and the residue chromatographed on silica (75% EtOH•NH_3/EtOAc) to afford 2-7.

^1H NMR (300 MHz, D_2O): δ 8.71(s, 1H), 8.16 (s, 1H), 7.71 (d, J = 6 Hz, 1H), 7.64 (d, J = 6 Hz, 1H), 7.54 (t, J = 6 Hz, 1H), 7.54 (t, J = 6 Hz, 1H), 7.08 (d, J = 7Hz, 1H), 6.06 (d, J = 7Hz, 1H), 5.35 (m, 1H), 4.81 (s, 2H), 3.53 (q, J= 6Hz, 2H), 3.43 (m, 2H), 3.00 (m, 2H), 2.69 (t, J = 6Hz, 2H), 2.60 (t, J = 7Hz, 2H), 2.46 (t, J = 6Hz, 2H), 2.25 (t, J = 7Hz, 2H), 2.05-1.90 (m, 4H).

EXAMPLE 3

Tablet Preparation

Tablets containing 25.0, 50.0, and 100.0 mg., respectively, of the following active compound are prepared as illustrated below:

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine.

TABLE FOR DOSES CONTAINING FROM 25-100MG OF THE ACTIVE COMPOUND

	Amount-mg		
Active Compound	25.0	50.0	100.0
Microcrystalline cellulose	37.25	100.0	200.0
Modified food corn starch	37.25	4.25	8.5
Magnesium stearate	0.50	0.75	1.5

All of the active compound, cellulose, and a portion of the corn starch are mixed and granulated to 10% corn starch paste. The resulting granulation is sieved, dried and blended with the remainder of the corn starch and the magnesium stearate. The resulting granulation is then compressed into tablets containing 25.0, 50.0, and 100.0 mg, respectively, of active ingredient per tablet.

EXAMPLE 4

Intravenous formulations

An intravenous dosage form of the above-indicated active compound is prepared as follows:

Active Compound	0.5-10.0mg
Sodium Citrate	5-50mg
Citric Acid	1-15mg
Sodium Chloride	1-8mg
Water for Injection (USP)	q.s. to 1 L

Utilizing the above quantities, the active compound is dissolved at room temperature in a previously prepared solution of sodium chloride, citric acid, and sodium citrate in Water for Injection (USP, see page 1636 of United States Pharmacopoeia/National Formulary for 1995, published by United States Pharmacopoeial Convention, Inc., Rockville, Maryland, copyright 1994.

EXAMPLE 5

Intravenous formulation

A pharmaceutical composition was prepared at room temperature using 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine, a citrate buffer, and

sodium chloride, to obtain a concentration of 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine of 0.25 mg/ml.

800 grams of water was introduced into a standard pharmaceutical mixing vessel. 0.25 grams of the ester was dissolved in the water. 2.7 grams sodium citrate and 0.16 grams citric acid were added to obtain a finished citrate concentration of 10 mM. 8 grams of sodium chloride was added. 200 grams of water was then added to achieve the desired final concentrations of ingredients. The resulting aqueous formulation had the following concentrations:

Ingredient	Amount
4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)-butanoyl-glycyl-3(S)-quinolin-3-yl-β-alanine	0.25 mg/ml
citrate buffer	10 mM
sodium chloride	8 mg/ml

The finished concentrated formulation is stored in a standard USP Type I borosilicate glass container at 30-40 degrees C. Prior to compound administration, the concentrated formulation is diluted in a 4:1 ratio resulting in a finished concentration of 0.05 mg/ml and transferred to an infusion bag.

Therapeutic Treatment

4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β–alanine may be administered to patients where inhibition of human or mammalian platelet aggregation or adhesion is desired.

The compound of the invention is useful in inhibiting platelet aggregation and thus, they may find utility in surgery on peripheral arteries (arterial grafts, carotid endaterectomy) and in cardiovascular surgery where manipulation of arteries and organs,

and/or the interaction of platelets with artificial surfaces, leads to platelet aggregation and consumption. The aggregated platelets may form thrombi and thromboemboli. The compound of the invention may be administered to these surgical patients to prevent the formation of thrombi and thromboemboli.

The compound of the invention is also effective as an inhibitor of osteoclast cellular adhesion, and can be administered to inhibit bone resorption.

The dosage regimen utilizing the compound of the present invention for this purpose is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

Oral dosages of the compound, when used to prevent osteoclast cellular adhesion, will range between about 0.01 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day and preferably 0.01-50 mg/kg/day and more preferably 0.01-20 mg/kg/day, e.g. 0.1 mg/kg/day, 1.0 mg/kg/day, 5.0 mg/kg/day, or 10 mg/kg/day. A once-a-day oral dosage is, for example, 10 mg, 100 mg, or 500 mg. Advantageously, the compound of the present invention may be administered in divided doses of two, three, or four times daily. Intravenously, the most preferred doses will range from about 1 to about 10 mg/kg/minute during a constant rate infusion. Furthermore, the compound can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, or course, be continuous rather that intermittent throughout the dosage regime.

BONE RESORPTION-PIT ASSAY

When osteoclasts engage in bone resorption, they will literally cause the formation of pits in the surface of bone that they are acting upon. Therefore, when testing compounds for their ability to inhibit osteoclasts, it is useful to measure the ability of osteoclasts to excavate these resorption pits when the inhibiting compound is present.

Consecutive 200 micron thick cross sections from a six mm cylinder of bovine femur diaphysis were cut with a low speed diamond saw (Isomet, Beuler, Ltd., Lake Bluff, Il). Bone slices were pooled, placed in a 10% ethanol solution and refrigerated until further use.

Prior to experimentation, bone slices were ultrasonicated twice, 20 minutes each in H_2O. Cleaned slices were placed in 96 well plates such that two control lanes and one lane for each drug dosage are available. Each lane represents either triplicate or quadruplicate cultures. The bone slices in 96 well plates were sterilized by UV irradiation. Prior to incubation with osteoclasts, the bone slices were hydrated by the addition of 0.1 ml Medium 199, pH 6.9 containing 15% fetal bovine serum and 1% penicillin/streptomycin.

Osteoclasts were isolated from the long bones of 1 to 3 day old rat pups (Sprague-Dawley) by modifications of Chambers et al., (J. Cell. Science, 66:383-399). The resulting suspension (0.75 ml/bone) was gently triturated 90-120 times using a wide bore transfer pipet. The cellular population was separated from bone fragments by a cell strainer with a 100 micron nylon mesh. 100 µl of the cell suspension was placed onto each bone slice. Test compound was then added at the desired experimental concentrations.

Bone slices exposed to osteoclasts for 20-24 hrs were processed for staining. Tissue culture media was removed from each bone slice. Each well was washed with 200 µl of H_2O, and the bone slices were then fixed for 20 minutes in 2.5% glutaraldehyde, 0.1 M cacodylate, pH 7.4. After fixation, any remaining cellular debris was removed by 2 min. ultrasonication in the presence of 0.25 M NH_4OH followed by 2 X 15 min ultrasonication in H_2O. The bone slices were

immediately stained for 6-8 min with filtered 1% toluidine blue and 1% borax.

After the bone slices have dried, resorption pits were counted in test and control slices. Resorption pits were viewed in a Microphot Fx (Nikon) fluorescence microscope using a polarizing Nikon IGS filter cube. Test dosage results were compared with controls and resulting IC_{50} values were determined for each compound tested.

The appropriateness of extrapolating data from this assay to utility and use in mammalian (including human) disease states is supported by the teaching found in Sato, M., et al., Journal of Bone and Mineral Research, Vol. 5, No. 1, 1990. That article teaches that certain bisphosphonates have been used clinically and appear to be effective in the treatment of Paget's disease, hypercalcemia of malignancy, osteolytic lesions produced by bone metastases, and bone loss due to immobilization or sex hormone deficiency. These same bisphosphonates are then tested in the resorption pit assay described above to confirm a correlation between their known utility and positive performance in the assay.

EIB ASSAY

Duong et al., J. Bone Miner. Res., 8:S 378, describe a system for expressing the human integrin $\alpha_v\beta_3$. It has been suggested that the integrin is involved in the attachment of osteoclasts to bone matrix, since antibodies against the integrin, or RGD-containing molecules, such as echistatin (European Publication 382 451), can effectively block bone resorption.

Reaction Mixture:

1. 175 µl TBS buffer (50 mM Tris•HCl pH 7.2, 150 mM NaCl, 1% BSA, 1 mM $CaCl_2$, 1 mM $MgCl_2$).
2. 25 µl cell extract (dilute with 100 mM octylglucoside buffer to give 2000 cpm/25 µl).
3. ^{125}I-echistatin (25 µl/50,000 cpm) (see EP 382 451).

4. 25 µl buffer (total binding) or unlabeled echistatin (non-specific binding).

The reaction mixture was then incubated for 1 h at room temp. The unbound and the bound $\alpha_v\beta_3$ were separated by filtration using a Skatron Cell Harvester. The filters (prewet in 1.5% polyethyleneimine for 10 mins) were then washed with the wash buffer (50 mM Tris HCl, 1mM $CaCl_2/MgCl_2$, pH 7.2). The filter was then counted in a gamma counter.

SPA ASSAY

Materials:

1. Wheatgerm agglutinin Scintillation Proximity Beads (SPA): Amersham
2. Octylglucopyranoside: Calbiochem
3. HEPES: Calbiochem
4. NaCl: Fisher
5. $CaCl_2$: Fisher
6. $MgCl_2$: SIGMA
7. Phenylmethylsulfonylfluoride (PMSF): SIGMA
8. Optiplate: PACKARD
9. 3-10 (see preparation below)(specific activity 500-1000 Ci/mmole)
10. test compound
11. Purified integrin receptor: $\alpha_v\beta_3$ was purified from 293 cells overexpressing $\alpha_v\beta_3$ (Duong et al., J. Bone Min. Res., 8:S378, 1993) according to Pytela (Methods in Enzymology, 144:475, 1987)
12. Binding buffer: 50 mM HEPES, pH 7.8, 100 mM NaCl, 1 mM Ca^{2+}/Mg^{2+}, 0.5 mM PMSF
13. 50 mM octylglucoside in binding buffer: 50-OG buffer

Procedure:

1. Pretreatment of SPA beads:
500 mg of lyophilized SPA beads were first washed four times with 200 ml of 50-OG buffer and once with 100 ml of binding buffer, and then resuspended in 12.5 ml of binding buffer.

2. Preparation of SPA beads and receptor mixture
In each assay tube, 2.5 µl (40 mg/ml) of pretreated beads were suspended in 97.5 µl of binding buffer and 20 µl of 50-OG buffer. 5 µl (~30 ng/µl) of purified receptor was added to the beads in suspension with stirring at room temperature for 30 minutes. The mixture was then centrifuged at 2,500 rpm in a Beckman GPR Benchtop centrifuge for 10 minutes at 4°C. The pellets were then resuspended in 50 µl of binding buffer and 25 µl of 50-OG buffer.

3. Reaction
The following were sequentially added into Optiplate in corresponding wells:

(i) Receptor/beads mixture (75 µl)

(ii) 25 µl of each of the following: compound to be tested, binding buffer for total binding or 3-8 (see preparation below) for non-specific binding (final concentration 1 µM)

(iii) 3-10 in binding buffer (25 µl, final concentration 40 pM)

(iv) Binding buffer (125 µl)

(v) Each plate was sealed with plate sealer from PACKARD and incubated overnight with rocking at 4°C

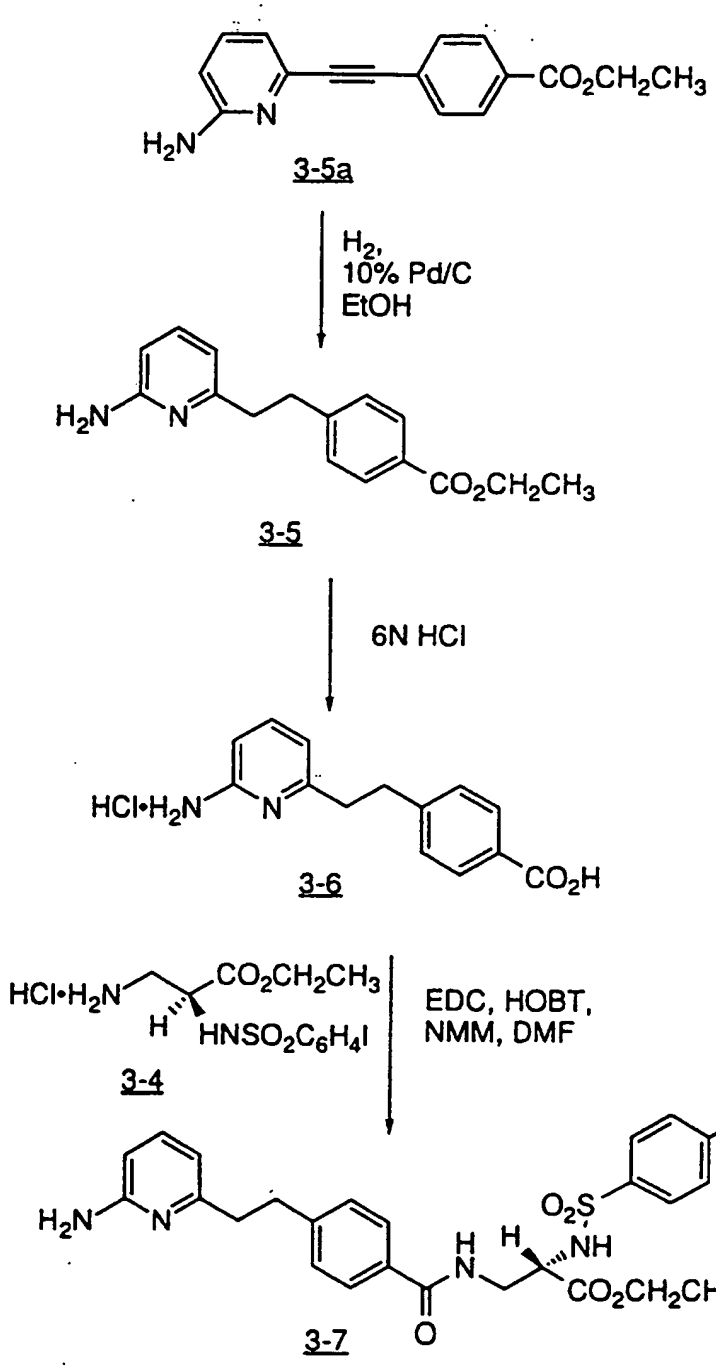
4. Plates were counted using PACKARD TOPCOUNT

5. % inhibition was calculated as follows:
A = total counts
B = nonspecific counts

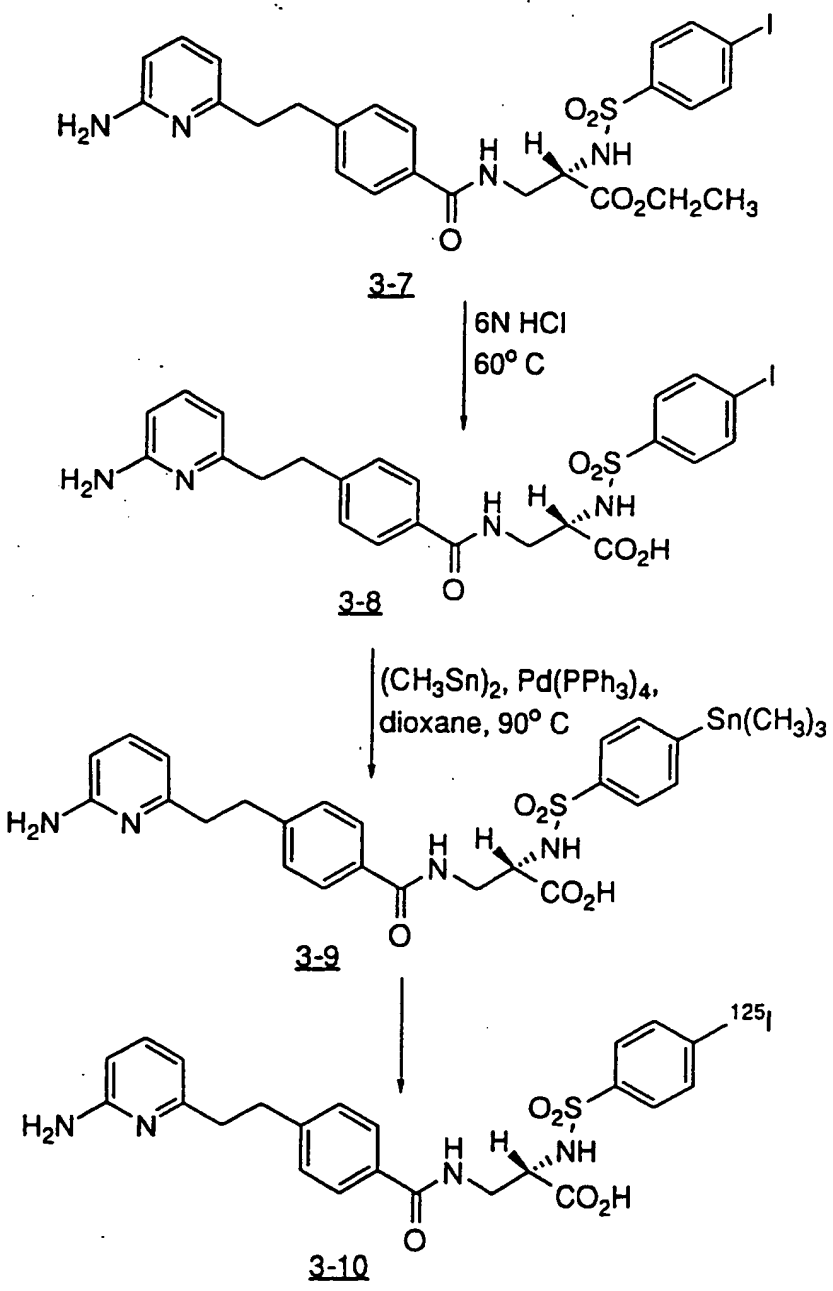
C = sample counts

% inhibition = [{(A-B)-(C-B)}/(A-B)]/(A-B) x 100

SCHEME 3 (CONT'D)



SCHEME 3 (CONT'D)



N-(4-Iodo-phenylsulfonylamino)-L-asparagine (3-2)

To a stirred solution of acid 3-1 (4.39 g, 33.2 mmol), NaOH (1.49 g, 37.2 mmol), dioxane (30 ml) and H_2O (30 ml) at 0°C was added pipsyl chloride (10.34 g, 34.2 mmol). After ~5 minutes, NaOH (1.49, 37.2 mmol) dissolved in 15 ml H_2O, was added followed by the removal of the cooling bath. After 2.0 h, the reaction mixture was concentrated. The residue was dissolved in H_2O (300 ml) and then washed with EtOAc. The aqueous portion was cooled to 0°C and then acidified with concentrated HCl. The solid was collected and then washed with Et_2O to provide acid 3-2 as a white solid.
^1H NMR (300 MHz, D_2O) δ 7.86 (d, 2H, J=8Hz), 7.48 (d, 2H, J=8Hz) 3.70 (m, 1H), 2.39 (m, 2H).

2(S)-(4-Iodo-phenylsulfonylamino)-β-alanine (3-3)

To a stirred solution of NaOH (7.14 g, 181.8 mmol) and H_2O (40 ml) at 0°C was added Br_2 (1.30 ml, 24.9 mmol) dropwise over a ten minute period. After ~5 minutes, acid 3-2 (9.9 g, 24.9 mmol), NaOH (2.00 g, 49.8 mmol) and H_2O (35 ml) were combined, cooled to 0°C and then added in a single portion to the reaction. After stirring for 20 minutes at 0°C, the reaction was heated to 90°C for 30 minutes and then recooled to 0°C. The pH was adjusted to ~7 by dropwise addition of concentrated HCl. The solid was collected, washed with EtOAc, and then dried in vacuo to provide acid 3-3 as a white solid.
^1H NMR (300 MHz, D_2O) δ 8.02 (d, 2H, J=8Hz), 7.63 (d, 2H, J=8Hz), 4.36 (m, 1H), 3.51 (dd, 1H, J=5Hz, 13Hz) 3.21 (m, 1H).

Ethyl 2(S)-(4-iodo-phenylsulfonylamino)-β-alanine-hydrochloride (3-4)

HCl gas was rapidly bubbled through a suspension of acid 3-3 (4.0 g, 10.81 mmol) in EtOH (50 ml) at 0°C for 10 minutes. The cooling bath was removed and the reaction was heated to 60°C. After 18 h, the reaction was concentrated to provide ester 3-4 as a white solid.
^1H NMR (300 MHz, CD_3OD) δ 7.98 (d, 2H, J=8Hz), 7.63 (d, 2H, J=8Hz), 4.25 (q, 1H, J=5Hz), 3.92 (m, 2H), 3.33 (m, 1H), 3.06 (m, 1H), 1.01 (t, 3H, J=7Hz).

Ethyl 4-[2-(2-Aminopyridin-6-yl)ethyl]benzoate (3-5)

A mixture of ester 3-5a (700 mg, 2.63 mmol), (for preparation, see: Scheme 29 of PCT International Application Publication No. WO 95/32710, published December 7, 1995) 10% Pd/C (350 mg) and EtOH were stirred under 1 atm H_2. After 20 h, the reaction was filtered through a celite pad and then concentrated to provide ester 3-5 as a brown oil.

TLC R_f = 0.23 (silica, 40% EtOAc/hexanes)

^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 2H, J=8Hz), 7.26 (m, 3H), 6.43 (d, 1H, J=7Hz), 6.35 (d, 1H, J=8Hz), 4.37 (m, 4H), 3.05 (m, 2H), 2.91 (m, 2H), 1.39 (t, 3H, J=7Hz).

4-[2-(2-Aminopyridin-6-yl)ethyl]benzoic acid hydrochloride (3-6)

A suspension of ester 3-5 (625 mg, 2.31 mmol) in 6N HCl (12 ml) was heated to 60°C. After ~20 h, the reaction was concentrated to give acid 3-6 as a tan solid.

^1H NMR (300 MHz, CD_3OD) δ 7.96 (d, 2H, J=8Hz), 7.80 (m, 1H), 7.33 (d, 2H, J=8Hz), 6.84 (d, 1H, J=9Hz), 6.69 (d, 1H, J=7Hz), 3.09 (m, 4H).

Ethyl 4-[2-(2-Aminopyridin-6-yl)ethyl]benzoyl-2(S)-(4-iodo-phenylsulfonylamino)-β-alanine (3-7)

A solution of acid 3-6 (400 mg, 1.43 mmol), amine 3-4 (686 mg, 1.57 mmol), EDC (358 mg, 1.86 mmol), HOBT (252 mg, 1.86 mmol), NMM (632 µl, 5.72 mmol) and DMF (10 ml) was stirred for ~20 h. The reaction was diluted with EtOAc and then washed with sat $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated. Flash chromatography (silica, EtOAC → 5% isopropanol/EtOAc) provided amide 3-7 as a white solid.

TLC R_f = 0.4 (silica, 10% isopropanol/EtOAc)

^1H NMR (300 MHz, CD_3OD) δ 7.79 (d, 2H, J=9Hz) 7.61 (d, 2H, J=8Hz), 7.52 (d, 2H, J=9Hz), 7.29 (m, 1H), 7.27 (d, 2H, J=8Hz), 4.20 (m, 1H), 3.95 (q, 2H, J=7Hz), 3.66 (dd, 1H, J=6Hz, 14Hz), 3.49 (dd, 1H, J=8Hz, 13Hz), 3.01 (m, 2H), 2.86 (m, 2H), 1.08 (t, 3H, J=7Hz).

4-[2-(2-Aminopyridin-6-yl)ethyl]benzoyl-2(S)-(4-iodophenyl-sulfonylamino)-β-alanine (3-8)

A solution of ester 3-7 (200 mg, 0.3213 mmol) and 6N HCl (30 ml) was heated to 60°C. After ~20 h, the reaction mixture was concentrated. Flash chromatography (silica, 20:20:1:1 EtOAc/EtOH/ NH_4OH/H_2O) provided acid 3-8 as a white solid.
TLC R_f = 0.45 (silica, 20:20:1:1 EtOAc/EtOH/NH_4OH/H_2O)
^1H NMR (400 MHz, DMSO) δ 8.40 (m, 1H), 8.14 (Bs, 1H), 7.81 (d, 2H, J=8Hz), 7.62 (d, 2H, J=8Hz), 7.48 (d, 2H, J=8Hz), 7.27 (m, 3H), 6.34 (d, 1H, J=7Hz), 6.25 (d, 1H, J=8Hz), 5.85 (bs, 2H), 3.89 (bs, 1H), 3.35 (m, 2H), 2.97 (m, 2H), 2.79 (m, 2H).

4-[2-(2-Aminopyridin-6-yl)ethyl)benzoyl-2(S)-(4-trimethylstannyl-phenylsulfonylamino-β-alanine (3-9)

A solution of iodide 3-8 (70 mg, 0.1178 mmol), ((CH3)3Sn)2 (49 µl, 0.2356 mmol), $Pd(PPh_3)_4$ (5 mg) and dioxane (7 ml) was heated to 90°C. After 2 h, the reaction was concentrated and then purified by prep HPLC (Delta-Pak C_{18} 15 µM 100A°, 40 x 100 mm; 95:5 → 5:95 H_20/CH_3CN) provided the trifluoroacetate salt. The salt was suspended in H_2O (10 ml), treated with NH_4OH (5 drops) and then lyophilized to provide amide 3-9 as a white solid.
^1H NMR (400 MHz, DMSO) δ 8.40 (m, 1H), 8.18 (d, 1H, J=8Hz), 7.67 (m, 5H), 7.56 (d, 2H, J=8Hz), 7.29 (d, 2H, J=8Hz), 6.95-7.52 (m, 2H), 6.45 (bs, 2H), 4.00 (m, 1H), 3.50 (m, 1H), 3.33 (m, 1H), 2.97 (m, 2H), 2.86 (m, 2H).

4-[2-(2-Aminopyridin-6-yl)ethyl]benzoyl-2(S)-4-^{125}iodo-phenylsulfonylamino-β-alanine (3-10)

An iodobead (Pierce) was added to a shipping vial of 5 mCi of $Na^{125}I$ (Amersham, IMS30) and stirred for five minutes at room temperature. A solution of 0.1 mg of 3-9 in 0.05 mL of 10% H_2SO_4/MeOH was made and immediately added to the $Na^{125}I$/iodobead vial. After stirring for three minutes at room temperature, approximately 0.04-0.05 mL of NH_4OH was added so the reaction

mixture was at pH 6-7. The entire reaction mixture was injected onto the HPLC for purification [Vydac peptide-protein C-18 column, 4.6 x 250 mm, linear gradient of 10% acetonitrile (0.1% (TFA):H_2O (0.1% TFA) to 90% acetonitrile (0.1% TFA):H_2O (0.1% TFA) over 30 minutes, 1 mL/min]. The retention time of 3-10 is 17 minutes under these conditions. Fractions containing the majority of the radioactivity were pooled, lyophilized and diluted with ethanol to give approximately 1 mCi of 3-10, which coeluted on HPLC analysis with an authentic sample of 3-8.

Instrumentation: Analytical and preparative HPLC was carried out using a Waters 600E Powerline Multi Solvent Delivery System with 0.1 mL heads with a Rheodyne 7125 injector and a Waters 990 Photodiode Array Detector with a Gilson FC203 Microfraction collector. For analytical and preparative HPLC a Vydac peptide-protein C-18 column, 4.6 x 250 mm was used with a C-18 Brownlee modular guard column. The acetonitrile used for the HPLC analyses was Fisher Optima grade. The HPLC radiodetector used was a Beckman 170 Radioisotope detector. A Vydac C-18 protein and peptide column, 3.9 x 250 mm was used for analytical and preparative HPLC. Solutions of radioactivity were concentrated using a Speedvac vacuum centrifuge. Calibration curves and chemical concentrations were determined using a Hewlett Packard Model 8452A UV/Vis Diode Array Spectrophotometer. Sample radioactivities were determined in a Packard A5530 gamma counter.

OCFORM ASSAY

Osteoblast-like cells (1.8 cells), originally derived from mouse calvaria, were plated in CORNING 24 well tissue culture plates in α MEM medium containing ribo- and deoxyribonucleosides, 10% fetal bovine serum and penicillin-streptomycin. Cells were seeded at 40,000/well in the morning. In the afternoon, bone marrow cells were prepared from six week old male Balb/C mice as follows:

Mice were sacrificed, tibiae removed and placed in the above medium. The ends were cut off and the marrow was flushed out

of the cavity into a tube with a 1 mL syringe with a 27.5 gauge needle. The marrow was suspended by pipetting up and down. The suspension was passed through >100 μm nylon cell strainer. The resulting suspension was centrifuged at 350 x g for seven minutes. The pellet was resuspended, and a sample was diluted in 2% acetic acid to lyse the red cells. The remaining cells were counted in a hemacytometer. The cells were pelleted and resuspended at 1 x 10^6 cells/mL. 50 μL was added to each well of 1.8 cells to yield 50,000 cells/well and 1,25-dihydroxy-vitamin D3(D3) was added to each well to a final concentration of 10 nM. The cultures were incubated at 37°C in a humidified, 5% CO_2 atmosphere. After 48 h, the medium was changed. 72 h after the addition of bone marrow, test compound was added with fresh medium containing D3 to quadruplicate wells. Compounds were added again after 48 h with fresh medium containing D3. After an additional 48 h the medium was removed, cells were fixed with 10% formaldehyde in phosphate buffered saline for 10 minutes at room temperature, followed by a 1-2 minute treatment with ethanol:acetone (1:1) and air dried. The cells were then stained for tartrate resistant acid phosphatase as follows:

The cells were stained for 10-15 minutes at room temperature with 50 mM acetate buffer, pH 5.0 containing 30 mM sodium tartrate, 0.3 mg/mL Fast Red Violet LB Salt and 0.1 mg/mL Naphthol AS -MX phosphate. After staining, the plates were washed extensively with deionized water and air dried. The number of multinucleated, positive staining cells were counted in each well.

<u>αvβ5 ATTACHMENT ASSAY</u>

Duong <u>et al.</u>, <u>J. Bone Miner. Res.</u>, 11:S 290, describe a system for expressing the human αvβ5.

Materials:

1. Media and solutions used in this assay are purchased from BRL/Gibco, except BSA and the chemicals are from Sigma.
2. Attachment medium: HBSS with 1 mg/ml heat-inactivated fatty acid free BSA and 2 mM $CaCl_2$.

3. Glucosaminidase substrate solution: 3.75 mM p-nitrophenyl-N-acetyl-beta-D-glucosaminide, 0.1 M sodium citrate, 0.25% Triton, pH 5.0.
4. Glycine-EDTA developing solution: 50 mM glycine, 5 mM EDTA, pH 10.5.

Methods:

1. Plates (96 well, Nunc Maxi Sorp) were coated overnight at 4 °C with human vitronectin (3 ug/ml) in 50 mM carbonate buffer (pH 9/.6), using 100 µl/well. Plates were then washed 2X with DPBS and blocked with 2% BSA in DPBS for 2h at room temperature. After additional washes (2X) with DPBS, plates were used for cell attachment assay.
2. 293 (alpha v beta 5) cells were grown in MEM media in presence of 10% fetal calf serum to 90% confluence. Cells were then lifted from dishes with 1X Trypsin/EDTA and washed 3X with serum free MEM. Cells were resuspended in attachment medium (3 X 10^5 cells/ml).
3. Test compound was prepared as a series of dilutions at 2X concentrations and added as 50 µl/well. Cell suspension was then added as 50 ml/well. Plates were incubated at 37 °C with 55 CO_2 for 1 hour to allow attachment.
4. Non-adherent cells were removed by gently washing the plates (3X) with DPBS and then incubated with glucosaminidase substrate solution (100 µl/well), overnight at room temperature in the dark. To quantitate cell numbers, standard curve of glucosaminidase activity was determined for each experiment by adding samples of cell suspension directly to wells containing the enzyme substrate solution.
5. The next day, the reaction was developed by addition of 185 µl/well of gylcine/EDTA solution and reading absorbance at 405 nm using a Molecular Devices V-Max plate reader. Average test absorbance values (4 wells per test samples) were calculated. Then, the number of attached cells at each drug

concentration was quantitated versus the standard curve of cells using the Softmax program.

WHAT IS CLAIMED IS:

1. A compound which is 4-(1,2,3,4-Tetrahydro-1,8-naphthyridin-7-yl)butanoyl-glycyl-3(S)-quinolin-3-yl-β–alanine, and pharmaceutically acceptable salts thereof.

2. The compound of Claim 1, or a pharmaceutically acceptable salt thereof, for use in inhibiting the binding of fibrinogen to blood platelets, treating thrombus formation or embolus formation, preventing thrombus or embolus formation, inhibiting the aggregation of blood platelets, preventing platelet thrombosis, preventing thromboembolism, preventing reocclusion, inhibiting osteoclast cellular adhesion to mammalian bone surfaces, inhibiting the solubilization of mammalian bone minerals by osteoclast cells, inhibiting diabetic retinopathy, or inhibiting macular degeneration in a mammal.

3. Use of a compound of Claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inhibiting the binding of fibrinogen to blood platelets, treating thrombus formation or embolus formation, preventing thrombus or embolus formation, inhibiting the aggregation of blood platelets, preventing platelet thrombosis, preventing thromboembolism, preventing reocclusion, inhibiting osteoclast cellular adhesion to mammalian bone surfaces, inhibiting the solubilization of mammalian bone minerals by osteoclast cells, inhibiting diabetic retinopathy, or inhibiting macular degeneration in a mammal.

4. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound of Claim 1 and a pharmaceutically acceptable carrier.

5. A method for inhibiting the binding of fibrinogen to blood platelets in a mammal, comprising administering to the mammal a composition of Claim 4.

6. A method for inhibiting the aggregation of blood platelets in a mammal, by blocking fibrinogen from acting at its receptor site, comprising administering to the mammal a composition of Claim 4.

7. A method for inhibiting osteoclast cellular adhesion to mammalian bone surfaces comprising treating the mammal with a pharmacologically effective amount of a composition of Claim 4.

8. A method for inhibiting the solubilization of mammalian bone minerals by osteoclast cells in a mammal comprising treating the mammal with a pharmacologically effective amount of a composition of Claim 4.

9. A method of eliciting an $\alpha_v\beta_3$, $\alpha_v\beta_5$ or dual $\alpha_v\beta_3/\alpha_v\beta_5$ antagonizing effect in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of a composition of Claim 4.

10. The method of Claim 9, wherein the antagonizing effect is an $\alpha v\beta 3$ antagonizing effect.

11. The method of Claim 10 wherein the $\alpha v\beta 3$ antagonizing effect is selected from inhibition of: bone resorption, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth.

12. The method of Claim 11, wherein the $\alpha v\beta 3$ antagonizing effect is the inhibition of bone resorption.

13. The method of Claim 9, wherein the antagonizing effect is an $\alpha v\beta 5$ antagonizing effect.

14. The method of Claim 13 wherein the αvβ5 antagonizing effect is selected from inhibition of: restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth.

15. The method of Claim 9, wherein the antagonizing effect is a dual αvβ3/αvβ5 antagonizing effect.

16. The method of Claim 15, wherein the dual αvβ3/αvβ5 antagonizing effect is selected from inhibition of: bone resorption, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation or tumor growth.

17. A method of treating or preventing a condition mediated by antagonism of a vitronectin receptor in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the composition of Claim 4.

18. The method of Claim 17, wherein the condition is selected from the group consisting of osteoporosis and cancer.

19. A method of inhibiting bone resorption in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the composition of Claim 4.

20. A method of treating osteoporosis in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the composition of Claim 4.

21. A composition comprising a carrier suitable for topical ophthamological administration and between about 0.01-5% w/v of a compound of Claim 1 or pharmaceutically acceptable salt thereof or a prodrug thereof.

22. A method for administering a composition of Claim 21 to a patient in need of such compound comprising topically applying to the patient's eye an effective amount of the composition.

23. A method for inhibiting inhibiting diabetic retinopathy or macular degeneration in a patient comprising topically applying to the patient's eye a therapeutically effective amount of the composition of Claim 21.

The Patent Office

-SR-

Application No:	GB 9815170.7	**Examiner:**	William Thomson
Claims searched:	1-23	**Date of search:**	28 September 1998

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P): C2C (CKZ)

Int Cl (Ed.6): C07D 471/04

Other: ONLINE:CAS-ONLINE, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
	NONE	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.